



<b>DECLARATION OF JOSEPH FISHER UNDER 37 C.F.R. §1.131</b>	Application Number	09/293,670
	Confirmation Number	5176
	Filing Date	April 16, 1999
	First Named Inventor	Joseph Fisher
	Examiner	Teresa Wessendorf
	Group Art	1639
	Attorney Docket No.	RIGL-036CIP

This Declaration with the attached Exhibits are being submitted in conjunction with the Applicants' Response to the Office Action dated February 24, 2006.

I, Joseph Fisher, M.D. Ph.D. do hereby declare as follows.

1. I am listed as an inventor of the above-referenced patent application.
2. Between June and September, 1997, I was a Scientist at Rigel Pharmaceuticals, Inc. (hereinafter "Rigel"). During this time, I was part of a program focused on the discovery of intracellularly-active peptides. The strategy employed by this program involved infecting cells with a library of retroviral vectors encoding candidate peptides, and selecting cells with an altered phenotype using fluorescence activated cell sorting (FACS)-based methods. The idea of using more than five FACS parameters to identify retrovirally-delivered, intracellularly-active peptides was developed before July 31, 1997.
3. I understand that the claimed subject matter of the above-referenced patent application relates to screening methods that include sorting a population of retrovirally infected cells using at least five fluorescence activated cell sorting (FACS) parameters. I

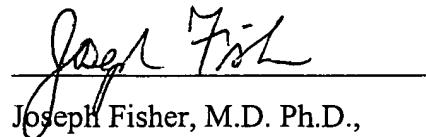
have been asked to provide factual evidence relating to my activities at Rigel with respect to the claimed subject matter before and after July 31, 1997.

4. Experiments confirming the applicability of FACS-based screening methods that employ at least five FACS parameters to the discovery of retrovirally-delivered intracellularly-active bioactive peptides were performed prior to July 31, 1997.
5. Exhibit A, which is a copy of pages 24 and 25 of my laboratory notebook, describes the results of an experiment in which cells were treated to induce exocytosis, and sorted using five FACS parameters. Exhibit A is dated prior to July 31, 1997. The top four graphs of page 25 show FACS results obtained from DMSO-treated cells (control), and the bottom four graphs of page 25 show FACS results obtained from A23187-treated cells (experimental). The top left graph of each group of four graphs shows results obtained from the parameter used to detect FM143, a fluorescent dye. The top right graph of each group of four graphs shows results obtained from the parameter used to detect FITC, another fluorescent dye. The bottom left graph shows results obtained from the parameter used to detect propidium iodide. The bottom right graph shows results obtained from parameter used to detect front light scatter as well as, independently, the parameter used to detect side light scatter. Thus, Exhibit A demonstrates the applicability of FACS methods that employ at least five FACS parameters to the discovery of retrovirally-delivered intracellularly-active bioactive peptides, before July 31, 1997.
6. Exhibit B, which is a copy of pages 112 to 120 of my laboratory notebook, describes an experiment in which and MC9 and CEM cells are transfected with a library of retroviral vectors that encode peptides. Exhibit B demonstrates that CEM and MC9 cells were transfected with a library of retroviral vectors between August 22 and August 27, 1997.

7. In September 1997, a method that included infecting cells with a library of retroviral vectors encoding candidate bioactive peptides, and selecting cells with an altered phenotype using five fluorescence FACS parameters was reduced to practice.
8. Exhibit C, which is a copy of pages 138 and 139 my laboratory notebook, describes an experiment in which retroviral vector library-infected cells are stimulated staurosporine to induce apoptosis, and sorted using five FACS parameters: side scatter ("ssc"), front light scatter ("fsc"), and three separate fluorescence parameters: ("fl1", "fl2" and "fl3"). Results for control cells not contacted with staurosporine are shown in the graphs on the left hand side of page 139, and results for experimental staurosporine-treated cells are shown in the graphs of the right hand side of page 139. Thus, Exhibit C demonstrates reduction to practice of a method that includes infecting cells with a library of retroviral vectors encoding candidate bioactive peptides, and selecting cells with an altered phenotype using five FACS parameters, on September 8, 1997.
9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: June 25, 2006

  
\_\_\_\_\_  
Joseph Fisher, M.D. Ph.D.,

Attachments: Exhibits A - C

HMC-1 - Encapsulation Trace Dyes/

# EXHIBIT A

- TM, FM-143 and Con A FITC as encapsulation Traces on HMC-1 cells.
- HMC-1 Cells - From Alex's spinner ~  $10^6$  cells/ml, Highly Viable
- Spin/Wash  $5 \times 10^6$  Cells in MT
- Dispense into 2-  $\frac{1}{2}$  Incubate in MT 10' 37°C
- " " " + Sucrose/Con A 100mg/ml  $\rightarrow$  37°C 10'
- Wash SCA Cells 2x MT
- Take up cells in 1ml MT (no BSA) in 4 TUBES
  - A) DMSO  $\rightarrow$  + FM-143- 2.5mg/ml  $\rightarrow$  37°C 10'
  - B) + A2387 1ug/ml  $\rightarrow$
  - C) DMSO  $\rightarrow$  + Con-A-FITC 25ug/ml  $\rightarrow$  "
  - D) A2387 "  $\rightarrow$

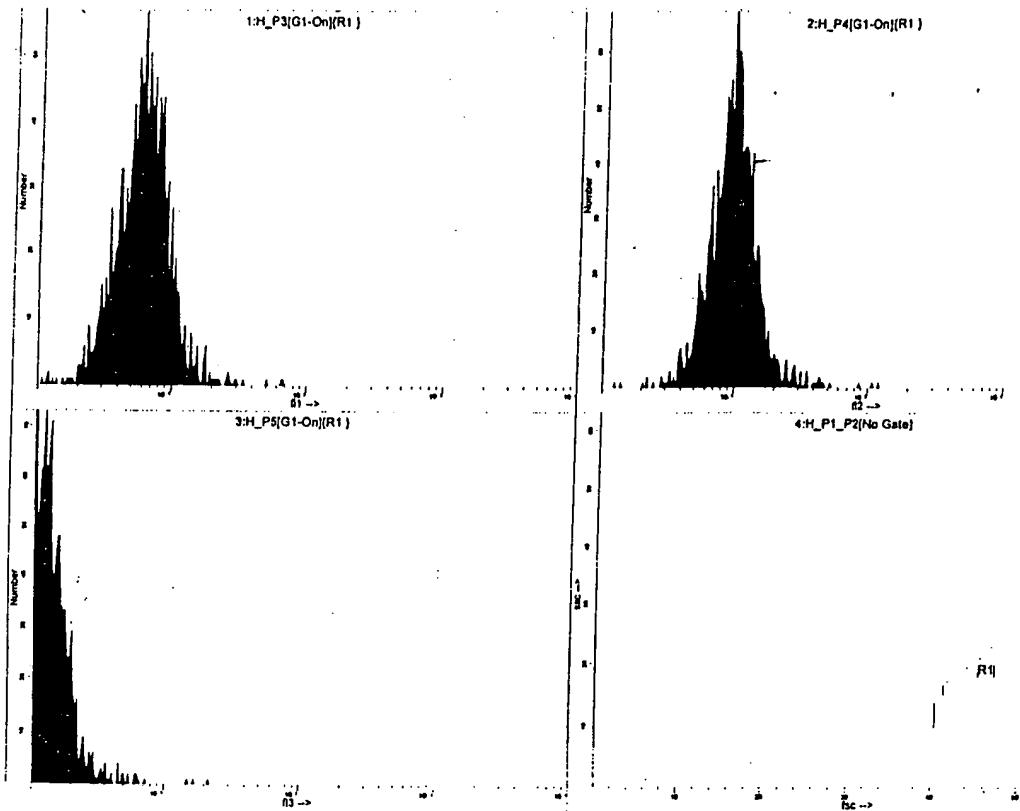
Wol Cells 1x in MT - Take up in 1ml for FACS

Save FACS as JMA012.001

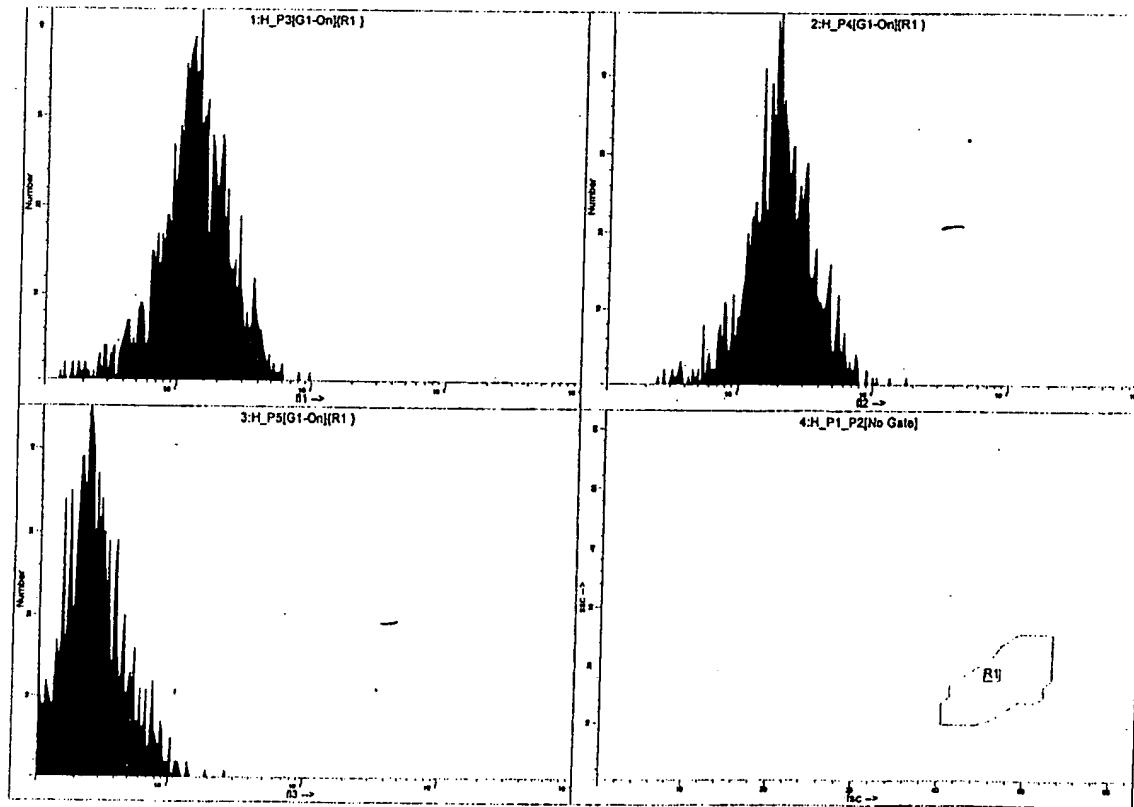
2	$\rightarrow$	D
3	$\rightarrow$	A
4	$\rightarrow$	B

To Page No. \_\_\_\_\_

Page No. \_\_\_\_\_



OMSP 10'  
37°C



1ug/ml  
A23187 10'  
37°C

To Page No. \_\_\_\_\_

Assed &amp; Understood by me,

Date

Invented by

Date

Recorded by

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

On Page No. \_\_\_\_\_

8/22/97

# EXHIBIT B

Phoenix E Cell Transfects  $\Rightarrow$  for MC9 Cell Infection

- Use Susans Protocol ( $\times 2$ ) So 2 wells of 6 well Plate / Transfection

- DNA - From Jenny Wang 1 (10μg) = 6.6λ Rab3a and Synaptotagmin

2 6.3λ Constructs

3 8.9λ

4 9.1λ

5 - New IRES Hook 43-13 129.13 10μg = 11.6λ  $\checkmark$  Randy's Nomenclature

6 - " " GFP 010-25 010-25 10μg = 11.1λ  $\checkmark$  Jim's Nomenclature

From  
Jim L

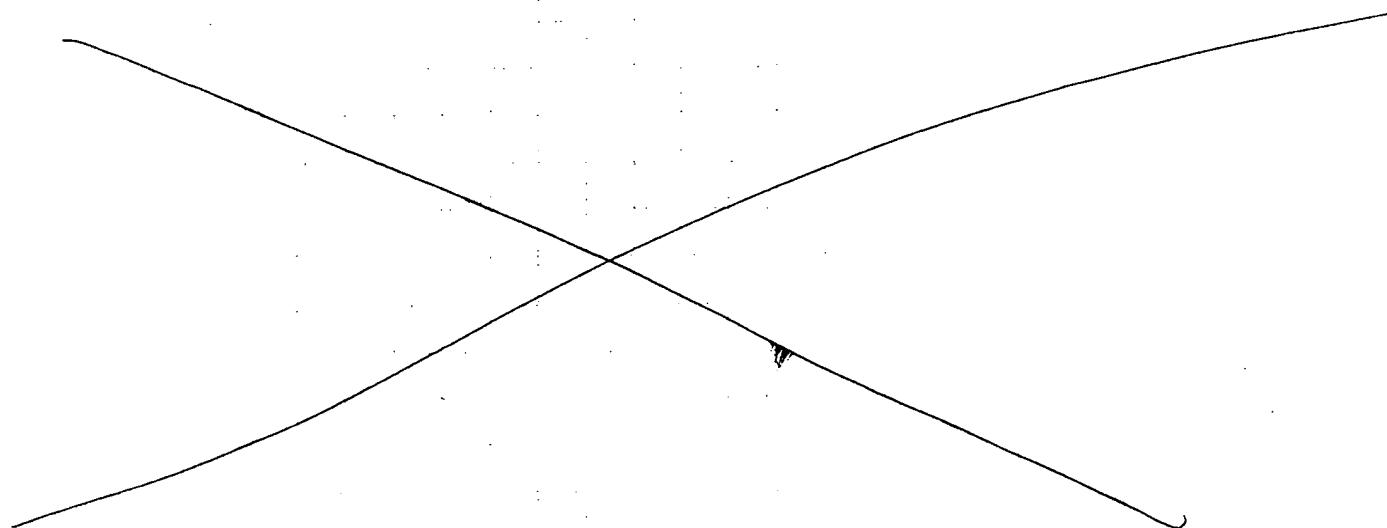
- Follow Susans Protocol - Put Preemptate / Chloroquine on cells at 11AM
- Micropipette & Preemptate Seen on all Transfectants

Protocol on next Page.

7PM  $\Rightarrow$  Aspirate DNA

- Use 1x1 Phoenix Media

- Add 2ml/well Fresh Media



On Page No. \_\_\_\_\_

Witnessed & Understood by me,  
Anne M. ...

Date

Invented by

W. M. ...

Signature

Date

8/22/97

Page No. \_\_\_\_\_

**Protocol for transfection of Phoenix cells and infection of nonadherent target cells****Day 1:**seed Phoenix cells (Es or As) in 6 well plates at  $8 \times 10^5$  cells in 1.5 ml (DMEM + 10% FBS + P/S) per well**Day 2: CaPO<sub>4</sub> Transfection**

per well:

5ug DNA  
30.5ul 2M CaCl<sub>2</sub>  
219ul H<sub>2</sub>O  
250ul 2X HBS2 wells10ug DNA  
61 $\lambda$  2M CaCl<sub>2</sub>  
438 $\lambda$  H<sub>2</sub>O  
500 $\lambda$  2X HBSallow all reagents to come to room temperature 30mins. before starting (do not warm up in H<sub>2</sub>O bath)

add 50mM chloroquine at 2ul/well (50um final)

**mix CaPO<sub>4</sub> reagents in 15ml polypropylene tube:**

pipet 5ug DNA to side of tube

pipet 30.5ul of 2M CaCl<sub>2</sub> away from the DNAmix the two together with the addition of 219ul of miliQ H<sub>2</sub>O

then using a 1ml pipet, add 250ul of 2X HBS and quickly bubble air through the pipet for 2 to 10 secs. (the time is 2 HBS batch dependent)

immediately add mixture dropwise to well

microscopically visible precipitate should appear within a few minutes

**incubate 8hrs**

remove medium, wash once, and replace with 1.5ml medium

**Day 3:**

move transfected plates to 32°C

**Day 4: Infection of target cells**

collect virus supernatent from transfected wells (1.5 ml) into 15 ml tubes and add either 1.5ul of 5mg/ml polybrene or 1.5ul 5mg/ml protamine sulfate

cfg out cells and debris at 2500 RPM for 5 mins. or alternatively, filter through .45um acrodisc syringe filter

count target cells and distribute  $5 \times 10^5$  cells per virus supe to 15ml tubes and pellet 5 mins. 2500 RPM

resuspend each pellet of target cells with virus supe and transfer to one well of a 24 well plate

seal plate with parafilm and cfg at RT for 90 mins. at 2500 RPM

Remove parafilm and incubate plate over night at 32°C

**Day 5:**

collect and pellet each well of target cells and resuspend in 4ml and transfer each to a 6cm plate

**Day 7 or Day 8:**

at 48 to 72 hrs. post infection target cells are ready to analyze for expression

To Page No. \_\_\_\_\_

Assessed &amp; Understood by me,

*Jamie Greene*

Date

*8/22/97*

Invented by

*Jay Frix*

Date

*8/22/97*

Page No. \_\_\_\_\_

8/23/97

- Transfections of  $\phi$ E Cells - (Cont.)
- This morning. 24 hrs post Transfection Start  
Look at Cells by Fluorescence.  
GFP + Cells Seen in # 3, 4, and 6  
3 and 4 must be CTIG Vector (inducible with Ires GFP)  
1 and 2 " be neg Hook vector.
- Remove old Media
- Add 2ml/ well of Warmed MC9 Media - 12PM

### (MC9 Positive Control Peptides)

MC9 Cells - WT

Scified Hook	} ~75% Hook +	From Amy.
Synaptotagmin		
RAB		

- " "

- " "

- " "

- Dispense 2ml Cells, Take up in .3ml MT  
100 $\mu$ l /Tube

⇒ one gets FM143 1ml

" " " "	+ 2ml Ionomycin	} 37°C → 30'
" " PI		

View in FACSCAN

- 001 WT
- 2 Hook
- 3 Synaptotagm.
- 4 RAB

- 5 - WT
- 6 + LWT
- 7 - HOOK
- 8 + "
- 9 - Synaptotagm.
- 10 + "
- 11 - RAB
- 12 + "

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

8/23/97

Invented by

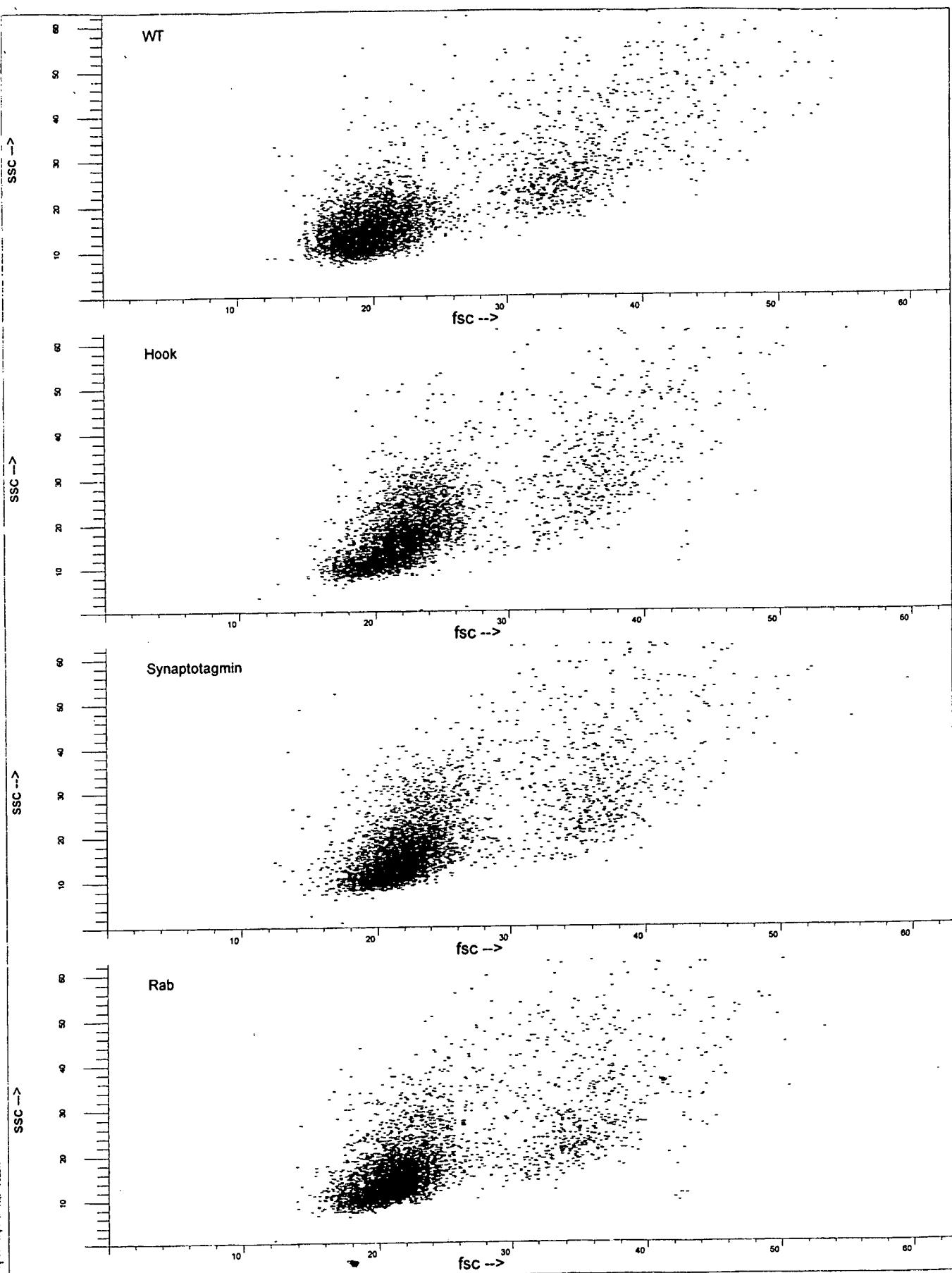
John

Fir

Date

8/23/97

Page No.



To Page No.

Assed &amp; Understood by me,

Date

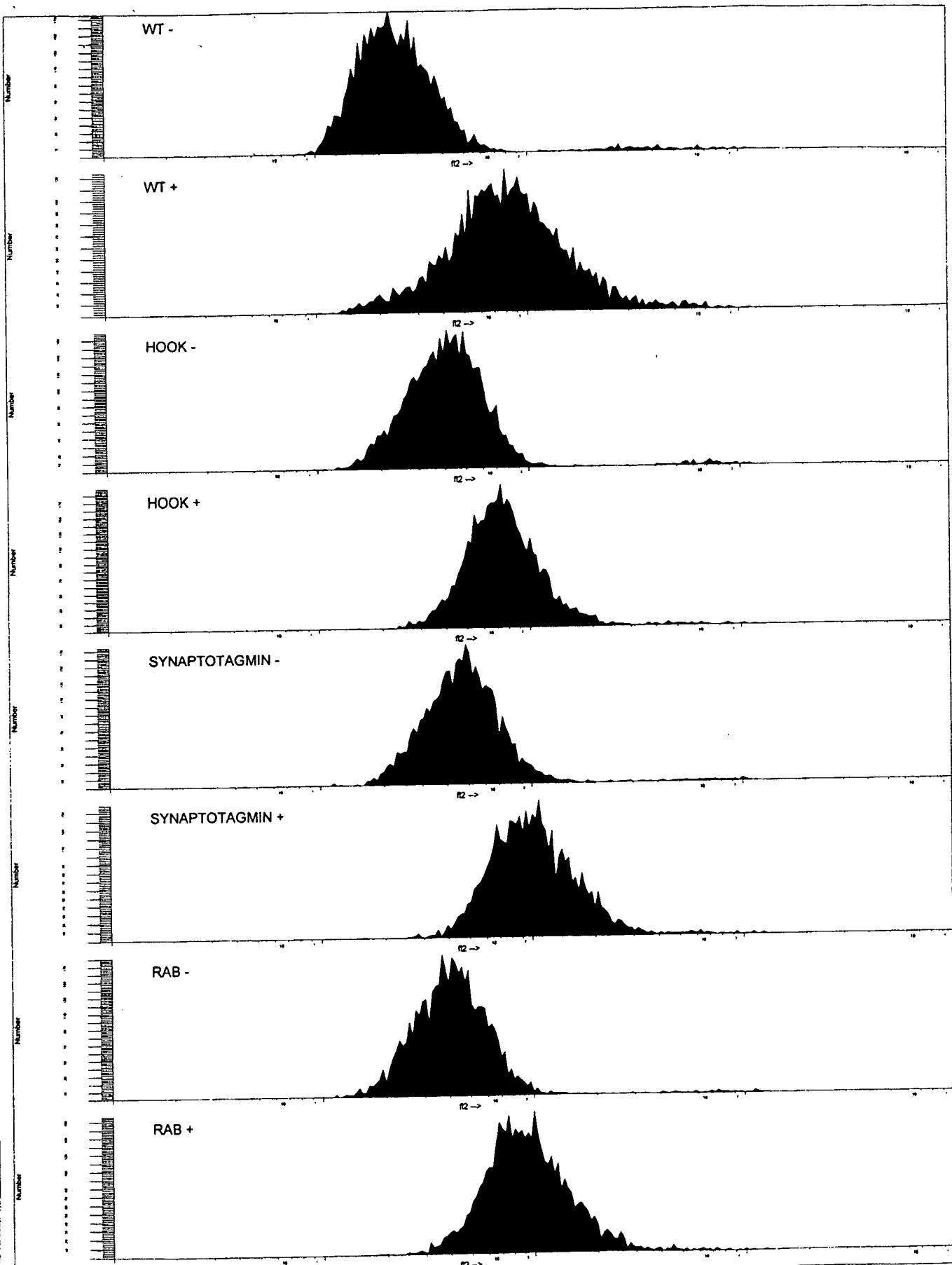
Invented by

Date

Project No. \_\_\_\_\_

6

om Pag



To Page No. \_\_\_\_\_

itnessed & Understood by me,

Date

10/10/03

Invented by

John FNL

Date

10/10/03

1 Page No. \_\_\_\_\_

[8/25]

= MC9 Cell Infection (Cont)

- wells 3/4 + 6 of Transfectors look significantly brighter for GFP than they did on 8/23
- ~1PM - Remove viral supernatant - Spin at 2500 RPM x 15' RT
- MC9 Cells,  $\sim 2.5 \times 10^6$  / ml
- Spin down 2 ml x 6 MC9 cells ( $\sim 5 \times 10^6$  / tube)
- Add viral supernatant
- Divide each into 2 wells of a 6 well plate ( $\sim 2$  ml /  $2.5 \times 10^6$  cells / well)  
Add 4  $\mu$ l of 5 mg/ml Polyamine Sulfate / well so FC = 10 mg/ml
- Seal plates and spin for 90' at 2500 RPM
- Culture overnight at 37°C ( $\sim 3:30$  PM → )

- MC9 Cell Harvest - for future cDNA Library Construction

Cells  $\sim 2 \times 10^6$  / ml

- Spin down 200 ml Cells

Wash 2x in cold PBS / Aspirate

- Freeze in dry ice - 2 tubes  $\times 2 \times 10^5$  cells / tube

- Store at -80°C

To Page No. \_\_\_\_\_

Assessed &amp; Understood by me,

Date

Invented by

Date

8/25/97

From Page No. \_\_\_\_\_

[8/26/97]

## + MC9 Infections (Cont.)

2x6 well Plates infected Yesterday

- ~ 11AM, Take Cells out of wells/ Pool, Wash Cells with 2ml MC9 Media, Spin, Decant
- Take up Pellets 1 to 6 with 12ml MC9 Media and Plate in T-75s
- Quick Look at #6 Showed some GFP  $\oplus$  Cells

## iresGFP Library Inf. Transfection

- Susan plated 20 60 mm Plates of  $\phi$ E Cells Yesterday, today ~ 40% Confluent
- Randy Supplied DNA 10-62 Library - 14mer, iresGFP 850  $\mu$ g/ml
- For Each 60mm Plate add (Plates have 6ml of Media)

82  $\lambda$  Chloroguanine (50mm)  
 10mg DNA (11.8  $\lambda$ )  
 122  $\lambda$  CaCl<sub>2</sub>  
 876  $\lambda$  H<sub>2</sub>O  
 1ml 2x HBS

Transfected 17 Plates From  
11:30AM  $\rightarrow$  ~12:30AM

## Collar Standard Procedure

~ 6:30PM

- Aspirate Media
- Wash Cells 1x in PBS + Catt
- Add Warm MC9 Media - 8ml/ Plate
- 37°C ~ 7PM

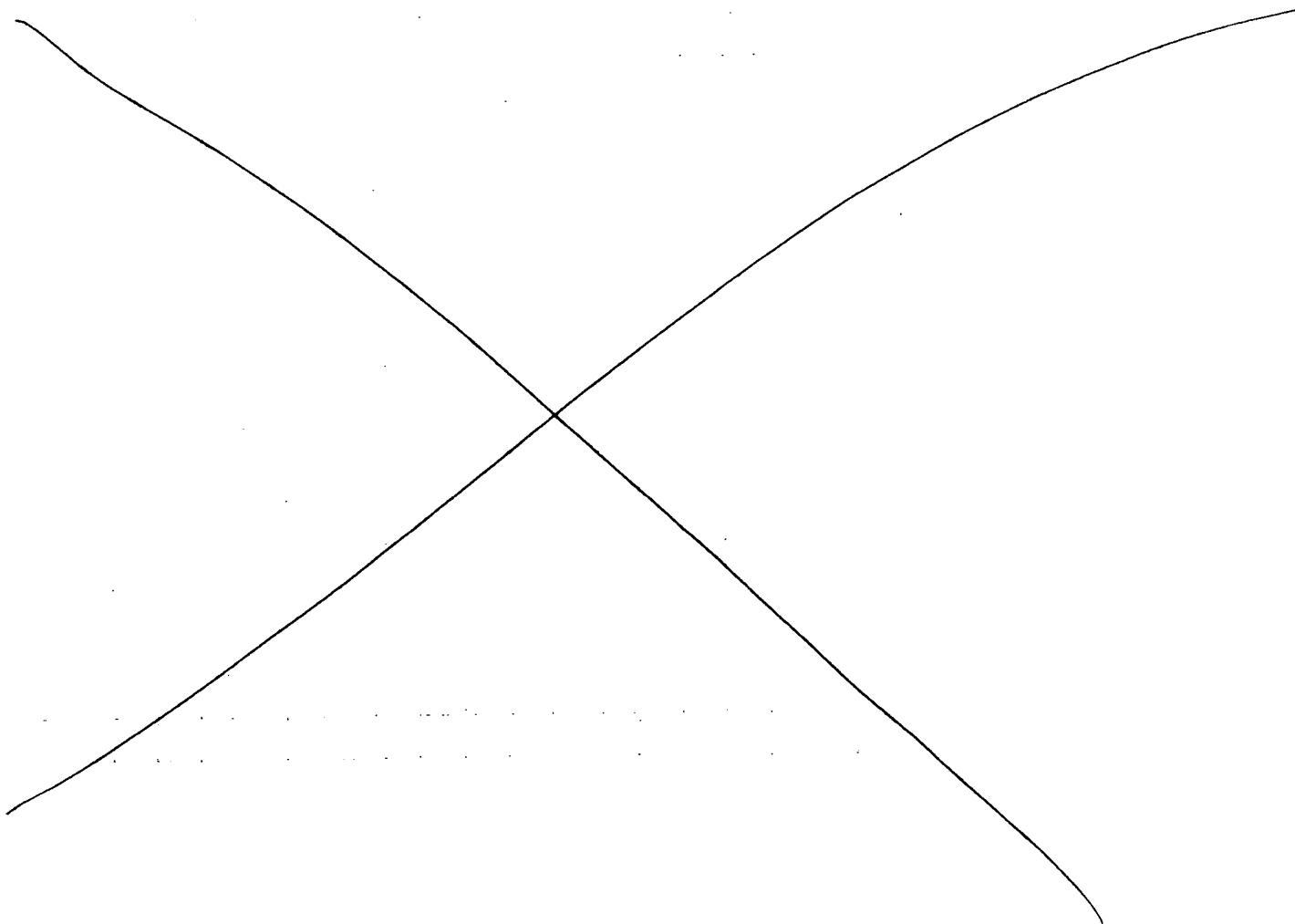
To Page No. \_\_\_\_\_

n Page No. \_\_\_\_\_

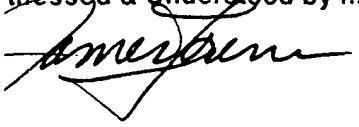
Library inires CFP,  $\sim 10^6$  Complexity of  
 random 14-mer Peptides - Part of 2nd  
 $\uparrow$  IgE Screen Library.

CEM - Library Infection

- Yesterday Susan S. Put CEM Media on Library Infected CFA Cells (After She Harvested her virus  $\sim$  3PM) - Today Remove Supers ( $\sim$  4PM) Spin at 2500 RPM  $\times 10'$ , Add PS to  $10\mu\text{g}/\text{ml}$
- CEM cells,  $\sim 1.1 \times 10^6/\text{ml}$   
 Spin Down 60ml ( $\sim 6.6 \times 10^7$  Cells Total)
  - Divide Pellets into  $8 \times 12\text{ml}$  Supers  $\Rightarrow$  / 8  $\times$  T-75S  $\Rightarrow$   $8.25 \times 10^6$  cells/Flask
  - Spin T-75S at 2500 RPM  
 $4:45 \rightarrow 6:15$
- Take out and Put at  $37^\circ\text{C}$  ON



To Page No. \_\_\_\_\_

Witnessed & Understood by me,	Date	Invented by	Date
	10/26/97	John Fox	8/26/97

In Page No. \_\_\_\_\_

[8/27/96]

- ΦE Library Transfection

A few GFP<sup>+</sup> cells seen today, but a minority  
 ~4PM Transfer cells to 32°C

- Split RCP cells for tomorrow's infection

[CEM Cells - Library Infection]

~1PM (22 hrs Post Infection Spin) Spin All infected CEM Cells - Decant Super.  
 Take up in 90ml Fresh Media  
 Plate in 3x T-150's

- Take out 1ml of Library Infected, 1ml of GFP Cells Annexin - PE / PI Stain as done on 8/13 (use those controls as well)  
 View in FACS CAN

Files 001 WT  
 002 Library Infected

→ See next page

Some GFP<sup>+</sup> cells Showing up in Library Infected after 22 hrs

Mod M9 Media

DMEM (has Pyruvate and Glutamine)

18mg/500ml Asparagine

1x Non-essential AA

0.05 mM 2ME

PenStrep 1x

10% HI FBS

10% T-Shift Conditioned Media

.22μm Sterile Filter

To Page No. \_\_\_\_\_

[9/8/97]

[CEM - Library Infected - Apoptosis Induction]

## EXHIBIT C

XX gave me Library Infected Cells to test DNA Rescue Methods - CEM  $\sim 2.4 \times 10^6$ /ml

Take 8ml ( $2 \times 10^7$  cells) + 4ml Fresh Media, Bring to 1ml Staurosporine

→ 37°C 10AM → 5PM (6 Hours)

→ Annexin PE Stain as usual Procedure = files

$\begin{cases} 015 & GFP \text{ ONLY} \\ 16 & Annexin PE \text{ ONLY} \\ 17 & PI \text{ ONLY} \\ 18 & GFP \text{ Library - } \emptyset \text{ Stavo} \\ 19 & " " + \text{Stavo 7 hrs} \end{cases}$

new settings

CEM-Library - Staurosporine treatment 2x (9/3) - Now 5 days post treatment

Take .5ml of culture - Add PI

- FACS CAN - .001 - Library untreated

.002 Treated Stavo 2x

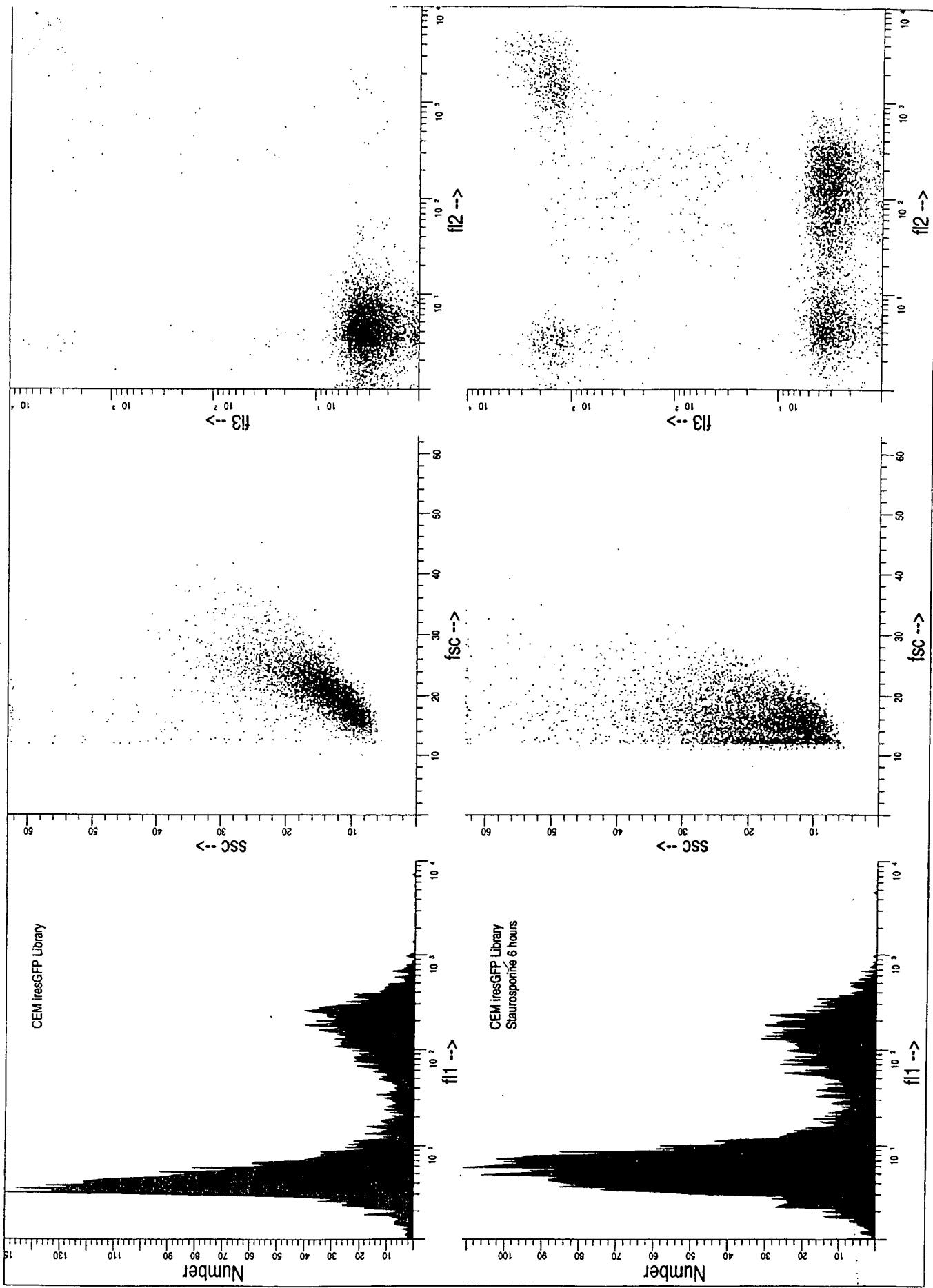
[MC9 Library - GFP Enriched]

- GFP Enriched Cells from last week - now  $\sim 2.8 \times 10^6$ /ml  $\times 100$ ml

- Split Back to  $\sim 10^6$ /ml for Tomorrow's Sort

- Remainder of Cells,  $\sim 2 \times 10^8$  cells

Spin / Decant, freeze in 5 vials ( $4 \times 10^7$ /vial) at -80°C



d & Understood by me,

Date

Invented by

Date

Understood by me  
James F. Green

Date

Invented by John F. F.  
Recorded by John F. F.

Date

— No. —

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record.**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**